REMARKS

Claims 1 to 34 are pending in this application.

Claim Rejections -35 U.S.C. §112

In paragraph 2, the Office rejected claims 1 to 31, 33 and 34 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In particular, the Office rejected the use of "a sample comprising said nucleic acid" and the narrower limitation "a sample consisting of said nucleic acid molecule" in claims 1 and 12.

In response, applicants have amended the claims to remove the narrower limitation.

In claim 34, the Office rejected the phrase "*wherein an allele frequency of 5% can be detected.*"

In particular, the Office expressed the opinion that the limitation is not an active process step and therefore fails to further limit the method of claim 10.

Applicants respectfully submit that a limitation does not have to be an "active process step" to constitute a legitimate limitation of a method claim. Rather, applicants submit that the rejected limitation provides information about the sensitivity of the current method in an appropriate format. Applicants further submit that these types of limitations are not uncommon in the field of diagnostics. For example, United States Patent 7,091,047 (issued 08/15/2006) to Serrero claims a "method of measuring the concentration of GP88 in a biological fluid." Claim 2 refers to the method of claim 1 and adds the limitation "*wherein GP88 can be detected at a concentration as low as about 0.1 nanograms of GP88 per milliliter.*" [emphasis added]

Accordingly, consideration of claim 34 is respectfully requested.

Claim Rejections- 35 U.S.C. §103

In paragraph 3, the Office rejected claims 1 to 12 and 19 to 28 and 30 to 34 under 35 USC §103(a) as being obvious over Eads et al., NAR 28(8):e32 (i) to (viii) (2000) (hereinafter "Eads") in view of U.S. Patent 6,258,568 to Nyren et al. (hereinafter "Nyren").

The Office expressed the view that Eads teaches all elements of claim 1, but for a real time sequencing step. The Office states that it would have been obvious at the time the invention was made to modify the method of Eads by pyrosequencing to confirm the methylation rather than using genomic bisulfite sequencing.

Eads discloses the MethyLight method, which employs locus-specific PCR primers flanking an oligonucleotide fluorescent labeled probe (page (ii), top of right column). The extension of the PCR primers results in the degradation of the probe and the release of a fluorescence signal, which in turn allows quantification of the PCR product, via, e.g., real-time quantitative PCR. MethyLight involves "real-time" quantification, it does not provide any sequence information nor does it involve any sequencing, certainly no real-time sequencing. A graph illustrating an embodiment of Eads' MethyLight method is attached directly to this response.

Eads' also and independent of the MethyLight method, performs sequencing via bisulfite genomic sequencing for comparative purposes (see, e.g., Fig. 6). Here, the PCR products are cloned and the individual clones are sequenced (Fig. 6B).

The disclosure of, on the one hand a quantification method (MethyLight) and a sequencing method (bisulfite genomic sequencing) in Eads in combination with the disclosure of Nyren, led the Office to the conclusion that it would have been obvious for one skilled in the art to "have modified the method of Eads et al by using pyrosequencing to confirm the methylation rather than genomic bisulfite sequencing." (emphasis added).

It is clear that the comparative bisulfite genomic sequencing the Office refers to does not provide any real-time benefits at all. This is, in fact, an issue that Eads addresses. Eads states, in particular, that "methods used to analyze cytosine-5 methylation patterns require cumbersome manual techniques that employ gel electrophoresis, restriction enzyme digestion, radio labeled dNTPs or hybridization probes." (see abstract) However, her solution is the MethyLight method, not the method currently claimed. Thus, rather than suggesting the desirability of the present invention

supporting a *prima facie* case of obviousness, Eads proposes an alternative method that is based on a different principle. Eads, a person of at least ordinary skill in the art, does so despite the fact that Nyren's pyrosequencing method had been published well before the submission of Eads' paper (See for example International publication WO9828440 (Nyren's underlying PCT application)).

As mentioned above, Eads' confirmatory genomic bisulfite sequencing is a method that is performed subsequent (in this case) and independent of the MethyLight method. Applicants submit that the nature of the MethyLight method essentially demands such an independent sequencing. The attach diagram illustrates (see also discussion above) the MethyLight method itself leaves little room for sequencing, certainly no room for real-time sequencing.

Any modification of Eads' MethyLight method that would be required to render it compatible with Nygen's pyrosequencing method would change the principle of operation of Eads' invention. This, however, further negates the existence of a *prima facie* case of obvious (MPEP §2143.01, VI).

For the advantages of real-time sequencing *per se* over, e.g., bisulfite genomic sequencing, applicants direct the Office's attention also to the literature cited on page 15 of applicants' response of July 14, 2006 as well as applicants' argument in the paragraph bridging pages 14 and 15 of the same response.

Notable are also different statements by Eads. A none exhaustive list includes: "It should be emphasized that the MethyLight technique was not designed to yield high-resolution methylation information, such as the pattern information obtainable with bisulfite genomic sequencing." (emphasis added, see, page viii, first full paragraph, first four lines). Also, Eads states that "Unlike other techniques, the MethyLight assay is completed at the PCR step, without the need for further gel electrophoretic separation or hybridization." (page viii, second full paragraph, lines 3 to 6).

The above shows that the following limitation of applicants independent claim 1 is not made obvious by the combination of Eads and Nyren:

> "(c) real-time sequencing said amplified nucleic acid treated with said agent" (emphasis added)
> (Independent claims 12 and 32 contain similar language).

["*Real-time sequencing*" is a "*sequence analyses which allow specific sequencing, i.e. determination of the sequence of a nucleic acid molecule in real-time.*" (Paragraph bridging pages 7 and 8)]

In particular, applicants submit that there is no motivation, including no implicit motivation, in the prior art, in particular not in Eads, or in the knowledge generally available to one of ordinary skill in the art, to modify and combine the reference teachings as required for a prima facie case of obviousness (MPEP §2143, para. 1.).

With regard to claim 12, the Office stated that the claim language "a method for the diagnosis of a pathological condition or the predisposition for a pathological condition" merely sets forth the intended use or purpose of the claimed invention, but does not limit the scope of the claim (thus excluding it from consideration). Applicants note that claim 12 also specifies:

"detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample wherein a methylated or a not methylated nucleotide is indicative of a pathological condition or the predisposition for said pathological condition." [Emphasis added].

Similarly, in claim 32, the Office's attention is directed at paragraph e. of the claim.

With regard to claim 34 (detection of allele frequency), the Office expressed the opinion that the limitation is an inherent property of the method.

Applicants note that a rejection based on inherency must be supported by a rationale or evidence tending to show inherency (MPEP §2112, IV.). Applicants note that no such rationale or evidence is provided. In fact, it appears that the Office is basing the inherency determination on the presently claimed method rather than the prior art.

In paragraph 4, claims 13 to 16, 18 and 29 are rejected under 35 USC §103(a) as being obvious over Eads in view of Nyren and further in view of United States Patent No. 5,786,146 to Herman (hereinafter "Herman").

In paragraph 5, claim 17 is rejected as being obvious over Eads in view of Nyren and further in view of United States Patent Publication No. 2003/0232351 to Feinberg (hereinafter "Feinberg") (The reference to U.S. Patent No. 6,251,594 to Gonzalgo in connection with Eads is assumed to be a typographical error. However, for a full discussion of this reference, please see applicants' response of July 14, 2006).

The deficiencies of Eads and Nyren have been discussed above. Neither Herman nor Feinberg cure these deficiencies.

Applicants have shown above that independent claims 1, 12 and 32 are non-obvious in view of Eads when combined with Nyren. These claims should therefore be in condition for allowance. Claims 2 to 11, 13 to 31 and 33 to 34 that are dependent therefrom should also be in condition for allowance.

The undersigned sincerely urges the Office to call her at the number provided below to discuss any issues that might arise in the further prosecution of this case.

No fees are believed to be due with this response. However, the Commissioner is authorized to charge or credit deposit account no. 50-3135 as required.

Respectfully submitted,

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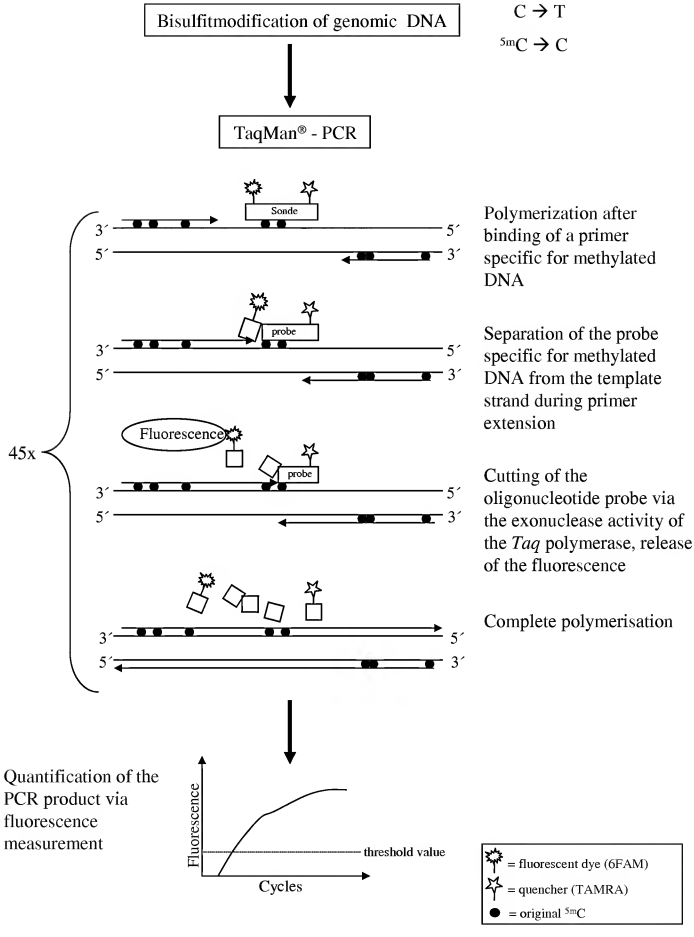
Bisulfitmodification of genomic DNA
C → T
5mC → C
TaqMan® - PCR
Sonde
3´
5´
probe
Fluorescence
45x
Polymerization after binding of a primer specific for methylated DNA
Separation of the probe specific for methylated DNA from the template strand during primer extension
Cutting of the oligonucleotide probe via the exonuclease activity of the Taq polymerase, release of the fluorescence
Complete polymerisation
Quantification of the PCR product via fluorescence measurement
Fluorescence
threshold value
Cycles
= fluorescent dye (6FAM)
= quencher (TAMRA)
= original 5mC